CLAIMS

We claim:

1. An isolated, pure population of mammalian CNS neuron-restricted precursor cells.

2. The population of claim 1 wherein said neuron-restricted precursor cells are capable of self-renewal.

3. The population of claim 1 wherein said neuron-restricted precursor cells are capable of differentiation to CNS neuronal cells but not to CNS glial cells.

4. The population of claim 1 wherein said neuron-restricted precursor cells express embryonic neural cell adhesion molecule.

5. The population of claim 4 wherein said neuron-restricted precursor cells do not express a ganglioside recognized by A2B5 antibody.

6. The population of claim 4 wherein said neuron-restricted precursor cells do not express nestin.

7. The population of claim 1 wherein said neuron-restricted precursor cells are selected from a mammalian embryo selected from the group consisting of human and non-human

primates, equines, canines, felines, bovines, porcines, ovines, lagomorphs, and the order Rodentia.

8. The population of claim 1 wherein said cells are able to differentiate into neurons that are capable of releasing and responding to neurotransmitters.

9. The population of claim 8 wherein said neurons demonstrate receptors for said neurotransmitters, and said cells are capable of expressing neurotransmitter-synthesizing enzymes.

10. The population of Claim 1 wherein said cells are capable of differentiating into neurons which can form functional synapses and/or develop electrical activity.

11. The population of claim 1 wherein said cells are capable of stably expressing at least one material selected from the group consisting of growth factors for said cells, differentiation factors for said cells, maturation factors for said cells, and combinations of any of these.

12. A method of isolating a pure population of mammalian CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of mammalian multipotent CNS stem cells capable of generating both neurons and glia;

(b) incubating the multipotent CNS stem cells in a medium configured for inducing said cells to begin differentiating;

(c) purifying from the differentiating cells a subpopulation of cells expressing a selected antigen defining neuron-restricted precursor cells; and

(d) incubating the purified subpopulation of cells in a medium configured for supporting adherent growth thereof.

13. The method of claim 12 wherein said selected antigen defining neuron-restricted precursor cells is embryonic neural cell adhesion molecule.

14. The method of claim 12 wherein said purifying comprises a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture.

15. The method of claim 12 wherein said procedure is specific antibody capture.

16. The method of claim 12 wherein said mammalian multipotent CNS stem cells are neuroepithelial stem cells.

17. The method of claim 16 wherein said isolating a population of CNS neuroepithelial stem cells comprises:

(a) removing a CNS tissue from a mammalian embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of cells in the neural tube;

(b) dissociating cells comprising the neural tube removed from the mammalian embryo;

(c) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuroepithelial stem cells comprising effective amounts of fibroblast growth factor and chick embryo extract; and

(d) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuroepithelial stem cells.

18. The method of claim 17 wherein said mammalian embryo is selected from the group consisting of human and non-human primates, equines, canines, felines, bovines, porcines, ovines, lagomorphs, and the order Rodentia.

19. The method of claim 17 wherein said substratum is selected from the group consisting of fibronectin, vitronectin, laminin, and RGD peptides.

20. The method of claim 12 wherein said medium comprises effective amounts of fibroblast growth factor and neurotrophin 3.

21. A method of isolating a pure population of mammalian CNS neuron-restricted precursor cells comprising the steps of:

(a) removing a sample of CNS tissue from a mammalian embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of glial and neuronal cells in the neural tube;

(b) dissociating cells comprising the sample of CNS tissue removed from the mammalian embryo;

(c) purifying from the dissociated cells a subpopulation expressing a selected antigen defining neuron-restricted precursor cells;

(d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(e) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells.

22. The method of claim 21 wherein said selected antigen defining neuron-restricted precursor cells is embryonic neural cell adhesion molecule.

23. The method of claim 21 wherein said purifying comprises a procedure selected from the group consisting of specific antibody capture, fluorescence activated cells sorting, and magnetic bead capture.

24. The method of claim 23 wherein said procedure is specific antibody capture.

25. The method of claim 21 wherein said mammalian embryo is selected from the group consisting of human and non-human primates, equines, canines, felines, bovines, porcines, ovines, lagomorphs, and the order Rodentia.

26. A pure population of mammalian CNS neuron-restricted precursor cells isolated by the method of claim 12.

27. A pure population of mammalian CNS neuron-restricted precursor cells isolated by the method of claim 21.

28. A method of obtaining postmitotic neurons comprising:

(a) providing neuron-restricted precursor cells and culturing the neuron-restricted precursor cells in proliferating conditions; and

(b) changing the culture conditions of the neuron-restricted precursor cells from proliferating conditions to differentiating condition, thereby causing the neuron-restricted precursor cells to differentiate into postmitotic neurons.

29. The method of claim 28 wherein said changing the culture conditions comprises adding retinoic acid to basal medium.

30. The method of claim 28 wherein said changing the culture conditions comprises withdrawing a mitotic factor from basal medium.

31. The method of claim 30 wherein said mitotic factor is fibroblast growth factor.

32. The method of claim 28 wherein said changing the culture conditions comprises adding a neuronal maturation factor to basal medium.

33. The method of claim 32 wherein said neuronal maturation factor is a member selected from the group consisting of sonic hedgehog, BMP-2, BMP-4, NT-3, NT-4, CNTF, LIF, retinoic acid, brain-derived neurotrophic factor (BDNF), and combinations of any of the above.

34. An isolated cellular composition comprising the mammalian CNS neuron-restricted cells of any of claims 1-7.

35. A pharmaceutical composition comprising a therapeutically effective amount of the composition of Claim 34 and a pharmaceutically acceptable carrier.

36. A method for treating a neuronal disorder in a mammal comprising administering to said mammal a therapeutically effective amount of the composition of Claim 34.

37. A method for treating a neuronal disorder in a mammal comprising administering to said mammal a therapeutically effective amount of the pharmaceutical composition of Claim 35.

38. The method of Claim 34 wherein said composition is administered by a route selected from the group consisting of intramuscular administration, intrathecal administration, intraperitoneal administration, intravenous administration, and combinations of any of the above.

39. The method of Claim 34 wherein said method also includes the administration of a member selected from the group consisting of differentiation factors, growth factors, cell maturation factors and combinations of any of the above.

40. The method of Claim 39 wherein said differentiation factors are selected from the group consisting of retinoic acid, BMP-2, BMP-4, and combinations of any of the above.

41. The composition of Claim 34 for use as a delivery vehicle for the delivery to glial cells of an agent selected from the group consisting of cell growth factors, cell maturation factors, cell differentiation agents, and any combinations of the above.

42. The composition of Claim 34 for use as a delivery vehicle for the delivery of trophic factors to neurons.

43. A method for treating neurodegenerative symptoms in a mammal comprising the steps of:

(a) providing a pure population of neuronal restricted precursor cells;

(b) genetically transforming said neuronal restricted precursor cells with a gene encoding a growth factor, neurotransmitter, neurotransmitter synthesizing enzyme, neuropeptide, neuropeptide synthesizing enzyme, or substance that provides protection against free-radical mediated damage thereby resulting in a transformed population of neuronal restricted precursor cells that express said growth factor, neurotransmitter, neurotransmitter synthesizing enzyme, neuropeptide, neuropeptide synthesizing enzyme, or substance that provides protection against free-radical mediated damage; and

(c) administering an effective amount of said transformed population of neuronal restricted precursor cells to said mammal.

44. A method or screening compounds for neurological activity comprising the steps of:

(a) providing a pure population of neuronal restricted precursor cells or derivatives thereof or mixtures thereof cultured *in vitro*;

(b) exposing said cells or derivatives thereof or mixtures thereof to a selected compound at varying dosages; and

(c) monitoring the reaction of said cells or derivatives thereof or mixtures thereof to said selected compound for selected time periods.

45. A method for treating a neurological or neurodegenerative disease comprising administering to a mammal in need of such treatment an effective amount of neuronal restricted precursor cells or derivatives thereof or mixtures thereof.

46. The method of claim 45 wherein said neuronal restricted precursor cells or derivatives thereof or mixtures thereof are caused to proliferate and differentiate *in vitro* prior to being administered.

47. The method of claim 45 wherein said neuronal restricted precursor cells or derivatives thereof or mixtures thereof are caused to proliferate *in vitro* prior to being administered, and then are caused to further proliferate and differentiate *in vivo* after being administered.

48. The method of claim 45 wherein said neuronal restricted precursor cells or derivatives thereof or mixtures thereof are caused to proliferate *in vitro* prior to being administered, and then are caused to differentiate *in vivo* after being administered.

49. The method of claim 45 wherein said neuronal restricted precursor cells or derivatives thereof or mixtures thereof are from a heterologous donor.

50. The method of claim 49 wherein said donor is a fetus.

51. The method of claim 49 wherein said donor is a juvenile.

52. The method of claim 49 wherein said donor is an adult.

53. The method of claim 45 wherein said neuronal restricted precursor cells or derivatives thereof or mixtures thereof are from an autologous donor.

54. The method of claim 53 wherein said donor is a fetus.

55. The method of claim 53 wherein said donor is a juvenile.

56. The method of claim 53 wherein said donor is an adult.

57. The method of claim 45 wherein said derivatives thereof are obtained by differentiation of neuronal restricted precursor cells *in vitro*.

58. The method of claim 45 wherein said derivatives thereof are obtained by genetic transduction of neuronal restricted precursor cells.

59. A method of isolating a pure population of mammalian CNS neuron-restricted precursor cells comprises the steps of:

(a) providing a sample of mammalian embryonic stem cells:

(b) purifying from the mammalian embryonic stem cells a subpopulation expressing a selected antigen defining neuron-restricted precursor cells;

(c) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(d) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells.